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RESEARCH ARTICLE

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Effect of four local anesthetics (tetracaine, bupivacaine, lidocaine and proparacaine) on intraocular pressure in rabbits- Comparison of an applanation and a rebound tonometer

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ABSTRACT

The type of device used, the type of local anesthetic agents, and the animal species may affect the intraocular pressure (IOP). Therefore, in order to determine these issues, the effects of four local anesthetics were investigated in 10 adult rabbits by ICare TA01i and Tono-Pen Vet tonometers. In the right eye of half of the rabbits and in the left eye of the other half of the rabbits, one drop of tetracaine was instilled. The IOP in each rabbit was measured using two tonometers, ICare and Tono-Pen Vet, before and each 5 minutes until 40 minutes later. The effects of other drugs were also studied at least with one-week interval. Based on the results of ICare tonometer, tetracaine significantly reduced the IOP immediately and 25 minutes after instillation. IOP changes after instillation of bupivacaine, lidocaine and proparacaine were not significant at any time compared to baseline values ($p > 0.05$). Based on the results of Tono-Pen Vet tonometer, all drugs reduced the IOP immediately after use; however, the effects of bupivacaine and lidocaine on IOP were much lower than that of tetracaine and proparacaine. The average duration of corneal anesthesia were 20, 15.5, 7.5 and 21 minutes for tetracaine, bupivacaine, lidocaine, and proparacaine, respectively. It is concluded that IOP reduction by local anesthetics when Tono-Pen Vet is used is much greater than the ICare tonometer measurements. Also, the reduction of IOP with each of the devices when tetracaine or proparacaine is used is greater than when bupivacaine or lidocaine is used.

Keywords

Bupivacaine, Intraocular pressure, Lidocaine, Proparacaine, Rabbit, Tetracaine

Abbreviations

IOP: intraocular pressure

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Introduction

Glaucomas are a group of eye diseases that commonly affects the optic nerve head and are caused by various factors, especially the increase of intraocular pressure (IOP). Therefore, in many cases, the intraocular pressure should be measured to diagnose the glaucoma (1). In order to measure the intraocular pressure, it is often necessary to use topical anesthetics (2, 3). The use of topical anesthetics may affect the IOP. Many studies have shown that the use of topical anesthetic agents reduce the IOP (4-8). On the other hand, there are other studies that have reported opposite findings, and have shown that these drugs do not change the IOP (9-11). The causes of these differences can be due to many factors (12, 13). We have previously shown in a study that tetracaine reduced the IOP in healthy and glaucomatous rabbits (8). In that study, the reduction of IOP in glaucomatous rabbits was higher than healthy rabbits. Therefore, one of the causes of differences in reports is the initial amount of IOP. In another study, we investigated the effects of tetracaine, bupivacaine, lidocaine, and proparacaine on IOP in the dogs (14). In that study we used a rebound tonometer (TA01i tonometer, ICare, Finland) that registers the IOP with lower ranges than the other tonometers (15, 16). In that study, tetracaine and proparacaine decreased the IOP, but the effect of lidocaine and bupivacaine on IOP was not significant. Therefore, another cause of the differences in reports is the types of drugs used. We thought that the third major factor is the type of device used to measure the IOP. Therefore, in the present study, we investigated the effect of four local anesthetics (tetracaine, bupivacaine, lidocaine and proparacaine) on intraocular pressure in rabbits using two types of tonometers (rebound ICare and applanation Tono-Pen Vet). With this aim in mind, we asked whether the type of drug, the type of device and animal species are effective in changing the IOP. Also, the duration of anesthesia in rabbits was measured and compared with other studies.

Results

Tetracaine

The results of intraocular pressure measurements with the ICare rebound and Tono-Pen Vet tonometers after the tetracaine instillation are shown in Fig. 1. The ICare tonometer readings showed that, IOP was immediately decreased after the administration of drug so that it was significant at times zero ($p = 0.046$) and 25 after instillation ($p = 0.027$). On the other hand, the Tono-Pen Vet readings showed that, the tetracaine immediately reduced the IOP. This IOP reduction continued up to 15 minutes after drug instillation and then began to increase; however, it was significantly lower than pre-treated values up to 30 minutes after drug administration. When compared to control eyes, the intraocular pressure reduction in treated eyes was significant at times 15 until 30 minutes after drug instillation.

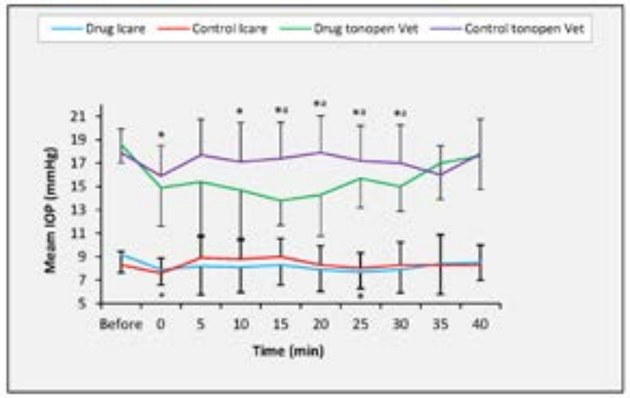

Figure 1.
Mean IOP of treated and control eyes in tetracaine group. The IOP in the treated eyes started to decrease immediately after instillation. The reduction of IOP by Tono-Pen Vet device was much sharper than the rebound Icare device; so that IOP by Tono-Pen Vet device decreased immediately after tetracaine instillation lasting for 30 minutes in treated eyes but by rebound Icare device, IOP reduction was significant only in 0 and 25 minutes after drug instillation. The data are based on the mean ± SD for 10 rabbits.
*: $p < 0.05$ comparing to pretreated baseline values.
a: $p < 0.05$ comparing to control eye values.

Bupivacaine

The results of intraocular pressure measurements using the ICare and Tono-Pen Vet tonometers after the bupivacaine administration are presented in Fig. 2. The ICare tonometer readings showed that the changes of IOP were not significant after the instillation of bupivacaine at any time compared to before baseline values; however when compared to control eyes, IOP reduction in the treated eyes was significant at times 10 ($p = 0.034$), 15 ($p = 0.040$), 20 ($p = 0.041$), and 25 ($p = 0.017$) minutes after drug administration. On the other hand, the results of Tono-Pen Vet tonometer indicated that the IOP significantly decreased at times 5 ($p = 0.011$), 10 ($p = 0.011$) and 15 ($p = 0.027$) minutes after instillation and then began to increase afterwards and reached its initial value in 40 minutes. Also, comparison of intraocular pressure in the treated eyes with the control eyes showed a significant decrease at 5 ($p = 0.016$), 10 ($p = 0.008$) and 15 ($p = 0.013$) minutes after drug instillation.

Lidocaine

As shown in Fig. 3, with the use of the ICare tonometer, the changes of IOP was not significant in the treated eyes after drug instillation compared to both baseline and control eyes ($p > 0.05$); but the Tono-Pen Vet tonometer readings showed that, the IOP significantly decreased 5 minutes after drug instillation compared to pretreated baseline values ($p = 0.021$). When compared to control eyes, the reduction of IOP in the treated eyes was significant at times 0 ($p = 0.024$) and 5 ($p = 0.007$).

Proparacaine

As shown in Fig. 4, using ICare tonometer, the IOP changes were minor and not significant. However, the differences of IOP in the treated eyes were significant at times 5 ($p = 0.017$), 15 ($p = 0.016$) and 20 ($p = 0.027$) compared to control eyes. On the other hand, by using Tono-Pen Vet tonometer, the IOP in the treated eyes started to decrease immediately after instillation of proparacaine and reduction of IOP was significant until 20 minutes after the administration of drug. The IOP in the treated eyes was significantly lower than that in control eyes immediately after drug instillation up to 25 minutes later.

Duration of anesthesia and side effects of drugs

All four drugs caused corneal anesthesia immediately after instillation. This effect was evaluated by corneal reflex, touching a piece of cotton with the cornea and seeing the animal's blink. Also, the returning of corneal sense was evaluated with this reflex. The mean duration of anesthesia was 20 minutes for tetracaine, 15.5 minutes for bupivacaine, 7.5 minutes for

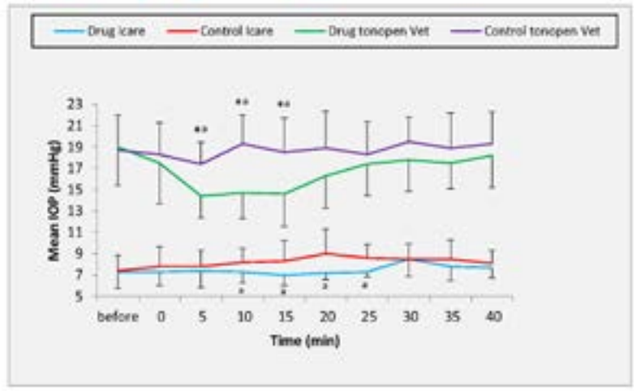

Figure 2.
Mean IOP of treated and control eyes in bupivacaine group. The IOP in the treated eyes started to decrease 5 minutes after instillation and lasting until 15 minutes after instillation by Tono-Pen Vet device. The IOP in the treated eyes by rebound Icare device were not changed comparing to pretreated baseline data. The data are based on the mean ± SD for 10 rabbits.
*: $p < 0.05$ comparing to pretreated baseline values.
a: $p < 0.05$ comparing to control eye values.

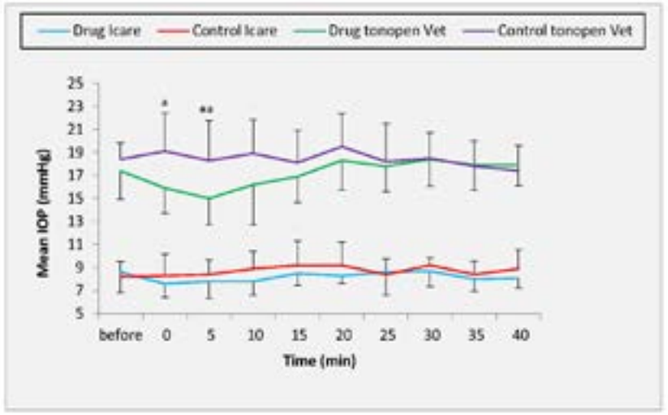

Figure 3.
Mean IOP of treated and control eyes in lidocaine group. The IOP in the treated eyes started to decrease immediately after instillation and was significant after 5 minutes by Tono-Pen Vet device. The IOP in the treated eyes by rebound Icare device were not changed ($p > 0.05$). The data are based on the mean ± SD for 10 rabbits.
*: $p < 0.05$ comparing to pretreated baseline values
a: $p < 0.05$ comparing to control eye values.

lidocaine, and 21 minutes for proparacaine. In this research, no adverse effects of drugs were observed in rabbits.

Discussion

Effects on IOP

Tetracaine

Sarchahi and bozorgi (2012), and Wang et al., (2013) evaluated the diurnal variation of IOP in rabbits, and reported that the intraocular pressure may change over the course of the day (8, 17). Thus, it is necessary to pay attention to this point and to investigate the effects of drugs in a short time. Therefore, in the present study, intraocular pressure in rabbits was

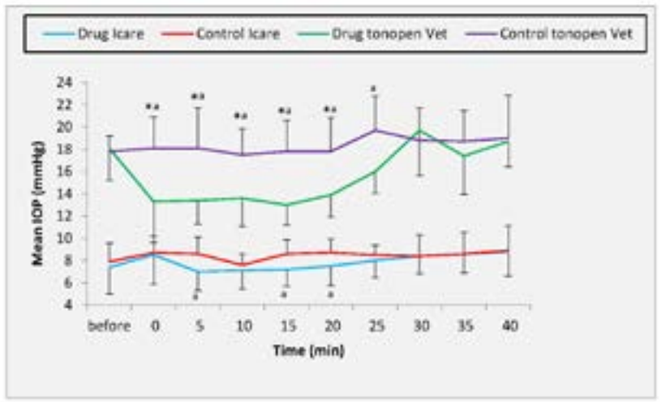

Figure 4.
Mean IOP of treated and control eyes in proparacaine group. The IOP by Tono-Pen Vet device decreased immediately after proparacaine instillation lasting for 20 minutes in treated eyes but by rebound Icare device, IOP reduction were not significant after drug instillation compared to baseline values. The IOP reduction by rebound Icare device was significant in 5 minutes after drug instillation lasting for 20 minutes compared to control eyes. The data are based on the mean ± SD for 10 rabbits.
*: $p < 0.05$ comparing to pretreated baseline values.
a: $p < 0.05$ comparing to control eye values.

taken from 13:00 to 18:00 h.

In the current study, the intraocular pressure, measured with both devices, has been reduced after instillation of tetracaine. The comparison of the IOP measured by two devices, Tono-Pen Vet and ICare, shows that firstly, the IOP measured with the Tono-Pen Vet is higher than that of the ICare device. Knollinger et al. (2005) and Leiva et al. (2006) comparing two Tono-Pen devices (Tono-Pen Vet, and Tonopen XL) and ICare in dogs and horses reported that the IOP shown by ICare was lower than that of Tono-Pen (15, 16). Secondly, because of the high pressure measured by the Tono-Pen Vet device, the effect of tetracaine on the IOP was more pronounced; While the IOP measured with ICare was lower and the reduction of IOP produced by this device was not very clear. The results of this study are almost the same as our two previous studies. In the first study, we compared the effects of tetracaine on healthy and glaucomatous rabbits and concluded that the greater the initial IOP, the more it decreases by tetracaine, so that the reduction of IOP in glaucomatous rabbits was more than healthy rabbits (8). In our previous second study, we investigated the effect of tetracaine on the intraocular pressure in dogs with a rebound ICare tonometer (14). In the previous and current studies, since the IOP measured by the ICare tonometer was low, the reduction in the IOP produced by tetracaine was low.

The results of this study and other researchers indicate that tetracaine reduces the IOP; however, the rate of reduction in IOP in various studies is different. One of the main causes of these differences appears to be the difference in baseline IOP before tetracaine instillation.

Bupivacaine

In a study done by Baudouin and Gastaud in the healthy and glaucomatous subjects, it was found that bupivacaine reduced the intraocular pressure within minutes 1, 5, and 15 after instillation (4). In our previous study, performed in dogs with ICare tonometer, bupivacaine did not significantly affect the IOP (14). In the present study, the results of the ICare device are consistent with our previous study. However, using of Tono-Pen Vet tonometer showed a decrease of IOP in 5-15 minutes. This finding is in agreement with the findings of Baudouin and Gastaud (4). This again emphasizes that the higher the initial IOP, the more it decreases, thus the decrease in IOP becomes significant. Nociti, et al., (2001) also reported a decrease in intraocular pressure 15 minutes after retrobulbar injection of bupivacaine. They have suggested that the reason of IOP decrease is the relaxation of extraocular muscles (18).

Lidocaine

There are some reports that show the using of lidocaine by other methods may affect the IOP; For example, Lerman and Kiskis (1985) and Abdulla and Flaifil (1991) reported that the use of lidocaine as an intravenous injection prevented the increase in IOP after tracheal intubation and laryngoscopy in children, and even 3 minutes after tracheal intubation, the IOP was also lower than that of zero time (19, 20). Hassanein et al. (2016) also reported similar results for lidocaine during the withdrawal of tracheal tubes (21). In the previous study, using the Icare tonometer, we concluded that lidocaine did not have a significant effect on IOP in dogs (14). In the present study, the effects of lidocaine on IOP were similar to those of ICare results and did not affect the IOP in rabbits; however when the IOP was measured with Tono-Pen Vet tonometer, it was found that lidocaine had some effect on the IOP; So that, IOP started to decrease immediately after administration and dropped to its lowest point within 5 minutes. Comparison of the effects of lidocaine with tetracaine and proparacaine in the present study showed that the reduction effect of lidocaine on IOP is similar to that of bupivacaine and is very low.

Proparacaine

The results of ICare tonometer showed that the proparacaine increased the IOP immediately after instillation (time 0), then IOP began to decrease and reached its lowest level in 5 minutes and then gradually increased. All these changes were not significant. On the other hand, results of Tono-Pen Vet tonometer showed that the IOP decreased immediately after instillation so that IOP was significantly lower than pretreated and control values until 20 and 25 minutes later respectively. Dosunmu et al. (2014), using ICare tonometer, evaluated the effects of 0.5% proparacaine on IOP in children (22). They reported that IOP slightly increased compared to before, and then, within 8 minutes after drug administration, a slight decrease in IOP was created, which, of course, was not significant. The results of ICare tonometer in the present study are similar to Dosunmu et al.'s study. Herse and Siu (1992), Ko et al., (2005) and Nam et al. (2006) reported that proparacaine causes a transient increase in the thickness of the cornea, thereby temporarily increases IOP (23-25). Leiva et al. (2006) Compared the IOP values of ICare and Tonopen XL tonometers in the eyes of healthy dogs (16). The results showed that ICare values were significantly lower than those of Tonopen XL ($p < 0.0001$), however, they concluded that the ICare tonometer could be an appropriate measurement method for daily clinical use after calibration for the dogs. As previously mentioned in this

discussion about tetracaine, the results of Leiva et al. and present study show that the IOP values obtained by the ICare tonometer are low. Therefore, the reduction effect of topical anesthetics such as proparacaine on IOP is less likely to be detected. Therefore, by measuring with this device, it seems that the drug has no effect on the IOP, but Tono-Pen Vet tonometer shows higher IOP values; As a result, the reducing effect of proparacaine on IOP is more visible.

Duration of anesthesia

In the present study, the average duration of corneal anesthesia after instillation of a drop tetracaine in rabbits was 20 minutes. This time has been reported 9.4, 16 and 30 minutes in humans, dogs and horses, respectively (14, 26, 27). Therefore, the duration of corneal anesthesia caused by tetracaine also vary in different species. We have already reported that the duration of corneal anesthesia were 20 and 22 minutes in healthy and glaucomatous rabbits, respectively (8). The findings of the present study confirm our previous findings on the duration of anesthesia in rabbits. Since two studies have been conducted in two separate geographical areas, the consistency of the results strongly confirms the effect of the species on the duration of corneal anesthesia.

The mean duration of corneal anesthesia after instillation of a drop bupivacaine in rabbits in the present study was 15.5 minutes. Sun et al. (1999) found that bupivacaine, and especially its buffered solution, had a greater effect than procaine or benzocaine on corneal anesthesia. The anesthetic effect of bupivacaine begins in the first minute after use, and if the acidity is adjusted, the duration of the effect becomes greater (28). In a study conducted by Liu et al. in rats, Bupivacaine had less toxic effects than proparacaine, and the duration of its effect is doubled by increasing the pH of the drug from 5.7 to 6.5 (29). In our previous study, the duration of corneal anesthesia by bupivacaine was 22 minutes in dogs (14). These findings indicate that the duration of corneal anesthesia caused by bupivacaine vary in different species, and in the present study, which is done on rabbits, it is less than the rest.

In the present study, lidocaine immediately after instillation caused corneal anesthesia but the mean duration of corneal anesthesia was very low (7.5 minutes). Assia, et al., (1999) concluded that lidocaine gel in human eye surgery was more effective than lidocaine drop, and had a good lubricating property (30). Shah et al. (2010) found that lidocaine (akten) gel produced longer anesthesia than lidocaine solutions in the eye and, due to containing of hydroxypropylcellulose, protects the corneal epithelium (31).

The duration of corneal anesthesia created by proparacaine varies in different species. Bartfield et al., in a study on humans, have shown that the degree and the duration of anesthesia created by proparacaine is greater than that of tetracaine (26). The results of the present study indicate that the duration of anesthesia created by proparacaine is longer than that of three other drugs. The reported duration of anesthesia induced by proparacaine in cats is 25 minutes (32), in dogs 45 and 34 minutes (14, 33), and in the horse 25 minutes (34).

Bupivacaine and lidocaine are amide anesthetics and they are classified as long-acting local anesthetic drugs. We expected their duration to be greater than tetracaine and proparacaine. But the duration of anesthesia caused by bupivacaine and lidocaine in the present study was in contrary to our expectations. One of the reasons for this can be the type of drug form used. In the present study, due to lack of topical forms of bupivacaine and lidocaine, we used an injection formulation of these drugs. This problem can be resolved in the future by producing and testing the topical form of these drugs. A second and more important reason can be the species in which the drug is used. As mentioned above, the effects of local anesthetic drugs vary in different animals and it seems that the effect of these drugs on rabbits is less than that of other species. Comparing the results of present study in rabbits with our previous study in dogs confirms this idea (14).

One of the limitations of the present study was that the injectable forms of these drugs were used. The second limitation was a small number of samples. However, this was a preliminary study, and these limitations can be overcome in the future by producing the topical form of these drugs and evaluating them in a larger number of samples.

It can be concluded that all drugs used in this study (tetracaine, bupivacaine, lidocaine and proparacaine) reduce the IOP immediately after use; however, the effects of bupivacaine and lidocaine on IOP were much lower than that of tetracaine and proparacaine. Thus, since they do not intensely affect the IOP and since lidocaine has antimicrobial and even positive effect on corneal cells (35), it is recommended that these two drugs be used topically before measuring IOP. Tetracaine and proparacaine reduce the IOP and this should be taken into account in the glaucomatous animals to avoid mistakes.

Material and methods

In the present study, 10 healthy adult white rabbits with a weight of 1.63-3.27 kg (mean±SD: 2.17 ±0.54) were used. Ages of rabbits were 6 months. Five male and five female rabbits were used. The study was approved by the research council of the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. The rabbits were kept in individual cages and were fed with dry commercial food and water ad libitum. All rabbits were carefully examined and were healthy, and showed no abnormalities in fluorescein staining, direct and panoptic ophthalmoscopy. Intraocular pressure of rabbits was measured at least once a day from a week before the start of the study to habituate the rabbits to this procedure.

To evaluate the effect of the first drug, one drop of 0.5% tetracaine (Anestocaine, Sina Darou, Tehran, Iran) was instilled in the right eye of five rabbits and the left eye of the other five rabbits. One drop of normal saline was instilled in the opposite eyes as controls. IOP was measured before and at 0, 5, 10, 15, 20, 25, 30, 35 and 40 minutes after drug instillation using an electronic rebound tonometer (TA01i tonometer, ICare, Finland) and immediately afterwards by an applanation tonometer (Tono-Pen Vet, Reichert, New York, USA). After an interval of at least one week, the effects of 0.5% bupivacaine (Marcaine® Spinal Heavy, Astrazeneca, Sweden), 2% lidocaine (Lignodig, Caspian Tamin, Iran) and 0.5% proparacaine (Alcaine, Alcon, Canada) were studied in the same way. Because IOP may vary throughout the day, IOPs were measured at 13:00–18:00 h in all rabbits. The sensation of the eyes was also examined every 5 minutes by corneal reflex (touching a piece of cotton with the cornea and observing the animal's blink). The rabbits were placed on a table in a relaxed state and prevented from any stress and minimal restraint was done on the head and neck without the use of systemic anesthetics or tranquilizers (Fig. 5). The eyelids were slowly opened and avoided any pressure on the eyelids and neck to prevent a change in IOP. Restraint was performed by the same assistant at all times. All measurements were also performed by a person who was unaware of the medication or placebo used in individual eyes and experienced with the use of both devices.

Statistical analysis: The normality of the data was analyzed using Shapiro-Wilk's statistical method. Since some of the data were abnormal, nonparametric tests were used for statistical comparisons. To compare the effect of each drug on time Friedman test, and in the case of significance, the Wilcoxon test was used to compare two sets of scores. The Wilcoxon test was also used to compare the IOPs of treated and control eyes. The Pearson correlation coefficient was used to test the relationship between IOP and the weight of rabbits. The Spearman correlation coefficient was used to determine the relationship between IOP and sex. The data are based on the mean ± SD for 10 rabbits. The level of significance was set at $p < 0.05$.

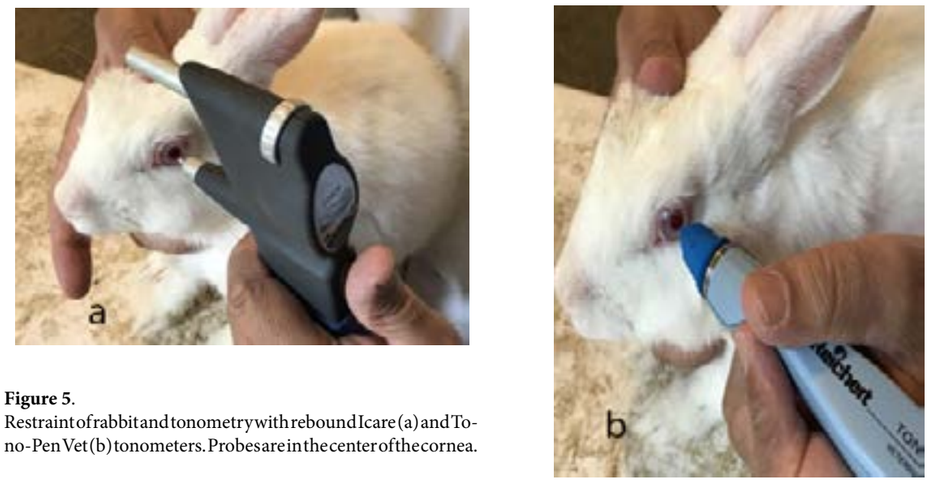

Figure 5.
Restraint of rabbit and tonometry with rebound Icare (a) and Tono-Pen Vet (b) tonometers. Probes are in the center of the cornea.

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Author Contributions

Design of Study: A.A.S, IOP measuring: A.A.S, A.E

Conflict of Interest

The authors declare that they have no conflict of interest.

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